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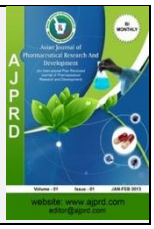

Open Access Research Article

A Research Article on Antistress Activity of Herbal Extract Oil of Piper Methysticum on Wistar Albino

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ABSTRACT

The research was carried out to determine the antistress activity of piper methysticum and the animal model used was wistar albino due to it's worldwide acceptability in researches related to psychology .kava (piper methysticum) root extract was used for abundance of kavlactones hence for the sake of study different percentage of suspensions like 4%, 6%, 8%, 12%, 18%, 30% were prepared and diazepam was selected as standard drug.oecd guideline 423 was followed during acute toxicity studies.no symptoms of acute toxicity was seen during 14 days observational period when given a dose of 2000 mg/kg so on this basis two dose level of 200mg/kg and 400 kg were selected for antistress activity.test model selected for antistress activity were forced swim test, tail suspension test and anoxia stress tolerance test.for respective tests a sharp decline in duration of immobility and increase in anoxia stress tolerance was seen.hence we can conclude that piper methysticum can play a significant role in the field of treating stress after futhur extensive clinical research.

Keywords- antistress, kava, oecd, wistar albino

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INTRODUCTION

It is has been reported that drugs with anti-stress properties induce a state of non-specific resistance against stressful conditions that causes emotional upheavals, hence they are recommended for a short periods of time to manage mental illnesses hence, meant for temporary use and should only be taken under strict medical supervision[1]. Amphetamine, caffeine and anabolic steroids are the most widely used drugs by people to combat stress however, there incidence of toxicity and dependence has limited there therapeutic usefulness against stressful events[2].

An answer to this perplexing problem of countering stress induced perturbations of physiological homeostasis came the plant kingdom. Herbal formulations have been in use for many years not only in Asian countries but also globally for human well-being[3]. The herbal formulations claimed to enhance physical endurance; mental functions and non-specific resistance of the body have been termed as "adaptogens"[4]. The actual word adaptogen was first used by a Soviet scientist, Dr. Nikolai Lazarev, who was funded by grants from the military was researching substances which produced a "state of nonspecific resistance" (SNIR)[5]. The potential utility of safer and cheaper herbal medicines as antistress agents have been reported as they can withstand stress without altering the physiological functions of the body.

Various herbs like Asparagus racemosus, Bacopa monnieri, Centella asiatica, Emblica officinalis, Hypericum perforatum, Matricaria recutita, Mentha piperita, Nepeta cataria, Ocimum sanctum, Passiflora incarnate, Piper methysticum, P. longum Tribulus terrestris, Valeriana officinalis and Withania somnifera are claimed to have immunomodulatory, adaptogenic and anabolic effects along with the ability to improve vital energy[6].

Medicinal Uses

Uses supported by clinical data

Short-term symptomatic treatment of mild states of anxiety or insomnia, due to nervousness, stress or tension[7].

Uses described in pharmacopoeias and in traditional systems of medicine

To induce relaxation, reduce weight and treat fungal infections[8].

Uses supported by experimental data

Effective as local anaesthetic, antispasmodic[8], musculorelaxant, antimycotic[9], sedative[10], anxiolytic, anticonvulsive, analgesic[11] and neuroprotective effects.

Uses described in folk medicine, not supported by experimental or clinical data

Treatment of asthma, common cold, cystitis, gonorrhoea, headaches, menstrual irregularities, urinary infections and warts , antidepressant[12].

Mechanism involved:

- Blockade of voltage-gated sodium channels .
- Enhanced interactions between ligand and corresponding receptors (e.g., amino butyric acid type A receptor)[13]
- Inhibition of enzyme activity (e.g., cyclooxygenase-2) and decrease in cytokine release (e.g TNF-α)[14].

4.0 MATERIALS AND METHODS

4.1 Collection Of Plant Extracts

The pure root extracts of *Piper methysticum* G.(Forst),Piperaceae was purchased from Shudhanta Herbal Products. B-147, Sector-6, Noida, U.P, India. The pure extract was volatile in nature, light brown in color with aromatic odor. Its sparingly soluble in distilled water.

Kava root extract

4.2 Drug and Chemicals

All the chemicals and reagents used were of analytical grade. The various reagents and solvents also was taken from Department of Pharmacy, Kota College of Pharmacy, Kota; such as Normal saline (0.9 gm of NaCl in 100ml distilled water). Formalin saline (10% v/v –10ml of formalin in 90 ml distilled water. Picric acid (for animals marking), Ethylene diamine tetra acetic acid (EDTA) (2% use in vial because of anticoagulant property). Prepare pure root extracts of Piper methysticum suspension in water with a variety of concentration including 4%, 6%, 8% 12% 18% and 30% was prepared by using tween -80 & span-80 used as the emulsifier. The standard and test drug used for this study are as follow.

Standard drug: Diazepam (1 mg/kg)

Test drug:*Piper methysticum* root Extract suspension

(100mg/kg, 200 mg/kg, and 400 mg/kg)

4.3 ACUTE TOXICITY STUDY

Acute toxicity studies were carried out for pure *Piper methysticum* root Extract suspension (PM Suspension) following (OECD guideline 423, 2001). Animals were divided in two group each having male rat (n=3) & kept for overnight fasting. Control group received normal saline, test group received pure Piper methysticum root Extract suspension in water was given orally in the dose of 2000 mg/kg body weight to test group. The animals were placed individually after dosing at least once during first 4th and daily thereafter for a total of 14 days. The changes in skin, fur, eyes, mucous membrane, respiratory, autonomic, and behavior pattern were noted.

4.3.1 Description of Method

4.3.1.1 Selection of animal species-The rodent species which are used in this project are S.D rats (150-200 g), normally males are used. Healthy young adult animals of commonly used laboratory strains should be employed. Each animal, at the commencement of its dosing, should be between 8-12 weeks old and its weight should fall in an interval within ± 20 % of mean weight of any previously dosed animals.

4.3.1.2 Housing and feeding condition-S.D rats weighing 150-200g for toxicity studies and Albino mice weighing 15-25g were used in the study. They were caged (22.5×37.5 cm) in a room under standard laboratory conditions i.e., temperature 23± 1ºc, relative humidity 55± 5% and lighting 12Hrs Light & 12 Hrs. Dark. The animals were fed on a palliated diet and water ad libitum. The animals were transfer to the laboratory at least 1 hr before the start of the experiment. The experiments were performed during the day (08:00-16:00hr). The ethical committee of the institute approved the protocol of the study. Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (IAEC/KCP/2021/07) and care of animals by CPCSEA and ethical norms was strictly followed during all experimental procedure .

4.3.1.3 Body weight-Body weight of treated rats were assessed during this 14-day observational period, once before commencement of dosing, once on 7th day & finally on 14th day using a sensitive digital balance. Any change in body weight of treated group was compared with control group

4.3.1.4 Relative organ weight-On 14th day of the observational period, all the animals were euthanized under chloroform anesthesia. Different organs the heart, liver, lungs, spleen, kidneys and brain were carefully dissected out, and weighed in grams (absolute organ

weight). The relative organ weight of each animals was then calculated & compared with control group,

4.3.1.5 Food and water intake measurement-Average food and water intakes were measured every day at the same hour during the observational period in control & treated groups.

4.3.1.6 Hematology-On the necropsy day, blood was withdrawn through snip cutting of the tail end of rat. The blood was placed into Anticoagulant (EDTA) bottles for hematological assay. Hb levels of blood samples were determined by the Sahli haemoglobinometer. RBC count was determined by the visual counting method. Packed cell volume (PCV), WBC (White blood cell), differential and platelet counts utilizing mechanical expansion and optical magnification, augmented by survival cell staining, the QBC II system driver's platelet count, WBC subgroups from linear measurement of the packed cell layers Buffy coat. The blood tube was centrifuged on the rotor of the QBC II centrifuge for 5 minute and read on the QBC II reader. MCV (Mean corpuscular volume), MCH (Mean corpuscular Haemoglobulin and MCHC (Mean corpuscular Haemoglobuin concentration) were calculated.

4.3.1.7 Histopathology-Three animals were selected randomly from each group, anesthetized with Chloroform and dissected through central abdominal incision. The kidneys, heart, liver sample were collected and immediately fixed in 10% saline- formalin in labeled sample plastic bottles. The tissue was dehydrated in graded concentration of Xylene, embedded in molten paraffin wax and sectioned at 5mu. Tissue sections were fixed on grease free glass side and stained with hematoxylene& eosin for light microscopy at 40X & 100X. Photomicrograph of some of the tissue was taken using a microscopic fitted with camera unit and processed in color photo laboratory.

4.4 ANTISTRESS ACTIVITY

4.4.1. Anoxia stress tolerance test[15]

Hermetic vessel(1L)

↓

Introduce the animal in vessel on 7^{th} , 14^{th} and 21^{st} days after treatment with drug

↓

Introduce stress

↓

Animal shows the first convulsions

↓

Immediately remove from the vessel and resuscitate (if needed). Delay in removal of animal may lead to death.

↓

Record the time duration of entry of animal into the vessel and appearance of the first convulsion – Anoxia tolerance time.

4.4.1. Forced swim test[16]

A polypropylene open cylindrical container (diameter 10 cm,height 25 cm), containing 15 cm of water at 25 ± 1 °C.

↓

On the 8^{th} day of dosing allow rats to swim till complete exhaustion

↓

Take the end point when animal ceases struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water.

↓

Animal said to be immobilized, the total duration of immobility during the 6-min test was scored.

↓

The duration of immobility was recorded and decrease in the duration of immobility during the FST was taken.

4.4.3. Tail suspension test[17]

Place the mouse inside a 3-sided cubicle

↓

Suspend its tail from a hanger attach to a linear load cell that measures activity

↓

Acclimate to testing conditions for at least 1 min, to account that animal are uniformly active during this time

↓

Measurements are taken for 7 min ,

↓

Exclude the mice that climb their tail or fell off the hanger from analysis

↓

Calculate the percent immobility by determining the time spent immobile during the last 6 min of the test, and report percent of time (on a scale of 0-1) spent immobile.

5. EVALUATION OF ANTISTRESS ACTIVITY

5.1. Grouping of Animals

Adult male albino mice of 20-25 gm were selected and divided into 5 groups of 5 animals in each group.

- Group I was Control group (animals only received normal saline).
- Group II treated with Standard drug Diazepam (1mg/kg).
- Group III treated with *Piper methysticum* root Extract(100 mg/kg).
- Group IV treated with *Piper methysticum* root Extract(200 mg/kg).
- Group V treated with *Piper methysticum* root Extract(400 mg/kg).

The oral route of administration was selected for the treatment of animals.

Figure 1: Oral route of drug administration

5.2 Forced Swim Test[16]-Forced swim test, the mainly commonly use behavioral form used for the showing antistress similar to action in rodents was planned by Porsolt. The method was similar as follow before. Mice was independently enforced to go swimming in open glass container (25cm x15 cm x 25) contain clean water to a altitude of 15cm and maintain at 26 degree. At this altitude of water and animals were not capable to hold up themselves by moving the base or the surface fortifications of container with their hind paw or tail. Water in the container was distorted following subjecting every animal to FST since use water has been to demonstrate the change actions (Sravaniet al., 2012). Every animal show vigorous faction during first 2 min phase of the experiment. The period of stillness was manually record for the duration of the then 4 min of the totality 6 min test stage (fig no.2).

5.3 Tail Suspension Test[17]-Tail suspension examination normally working behavioral model for showing antistress like action in rodents. The animal were motivated from home settlement to laboratory possess cage and allowable to adjust to laboratory situation for 1-2 hr. All animal were separately balanced to the border of bench, 50cm on top of the ground by adhesive tape located approx 1cm from the tip of the tail. All animal below test be together acoustically and visually remote from other animals for the duration of the test. The total phase of immobility was record manually for 6 min. Animal was measured to immobile while it did

not demonstrate any corpse movement, hang unreceptively and totally immobile. The test was conduct in not bright area and all mouse was used only once in the test (Rajesh et al., 2012), the observer records the immobility of animals was sightless to medicine treatment known to the animals in study (fig no.3).

5.4 Anoxia Stress Tolerance Test[15]-Swiss mice of each gender were separated into 5 groups, each group have 5 mice. Group 1st provide as normal saline, group 1st, 2nd, 3rdwere treat with uncontaminated *Piper methysticum* root Extract suspension (PM Suspension)at doses 100, 200, 400 mg/kg, p.o and stress. Group 5th mice were treating with diazepam (1mg/kg) and stress. The drug action was passed out daily for a stage of 21 days. At the finish of this week i.e.1st, 2nd, 3rd weeks of medicine treatment, the animal was showing to anoxia tolerance time was noted. Hermetic vessel of 1 liter air capability was use to persuade to anoxia stress. All animal was reserved in the hermetic vessel and the time to demonstrate the first signal of seizure was noted and was directly detached from the vessel and resuscitate if desired (Said et al., 2013) (fig no. 4).

Figure No. 2: Mice immobilized with open cylindrical vessel

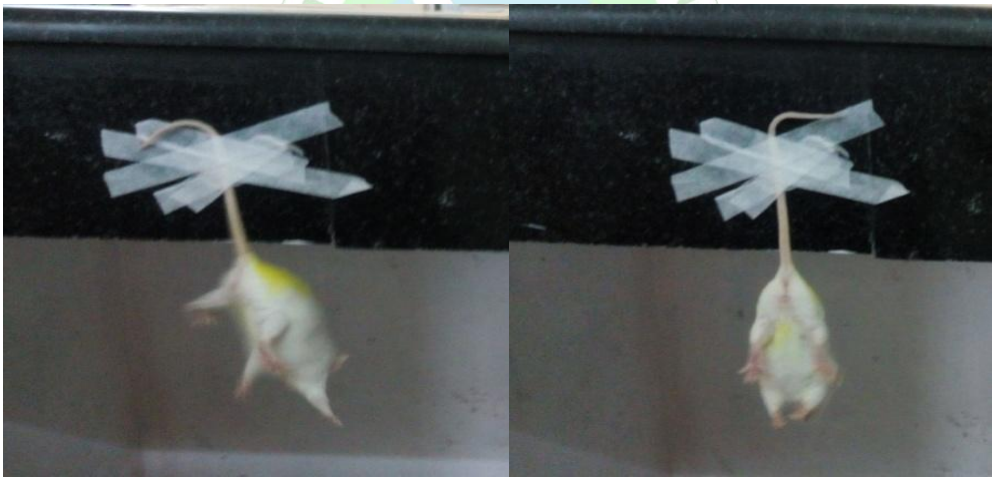


Figure No. 3: Mice immobilized with hanging tail

Figure No. 4: Induce stress with help of Hermetic vessel

6.1 ACUTE TOXICITY STUDY

According to OECD guideline 423 for acute oral toxicity at the dose of 2000 mg/kg, animal in the group treated with *Piper methysticum* root Extract suspension (PM Suspension)did not show any symptoms of toxicity at this dose level during the 14 days of observational period. At the dose level tested, no unwanted clinical signs were observed in the surviving rat. Here is no change in the behavior of stool, urine and eye color of all animals. All the treated animals had normal appearance & showed no abnormal activity. They all had a normal pattern of respiration. There was complete absence of symptom of sedation, convulsion and Lacrimation. No morbidity & mortality was observed in the treated group of rat. On the basis of these observations two dose levels 200 mg/kg & 400 mg/kg of *Piper methysticum* root Extract suspension (PM Suspension)were selected for Anti-stress activity.

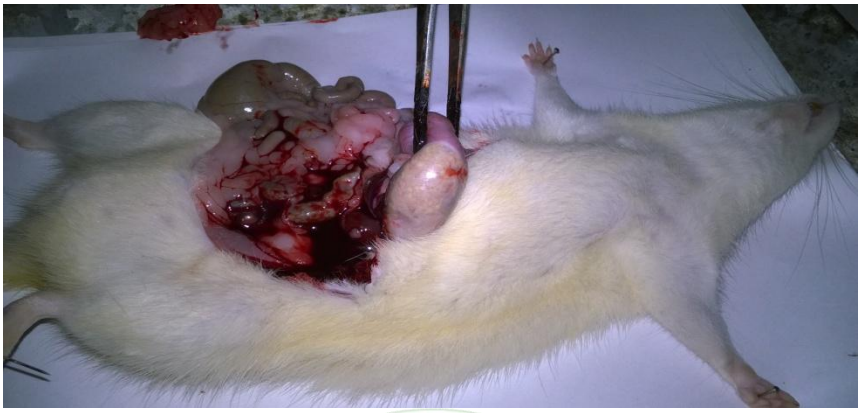


Figure no. 5: Autopsy animal for toxicities in organ

6.1.1 Body Weight Observation

From day 1 to 14 there were variable changes in the body weight of rat in both groups. The control rat gained weight throughout the duration of observation where as a slight decrease in weight was observed in rat treated with 2000 mg/kg of *Piper methysticum* root Extract suspension (PM Suspension)in last week of observation. All animals exhibited normal change in body weight without drastic difference between both and treated groups.

Table No. 1: Effect of PM root Extract suspension 2000 mg/kg on body weight

S.No.	Control			Treated		
	Initial	Middle	Final	Initial	Middle	Final
1.	162	170	181	163	170	175
2.	163	169	173	171	168	161
3	156	168	172	172	168	173
Mean	160±0.330	169±0331	175±0.332	168±0.334	169±0.331	169±0.321

Mean ± S.E.M. for n= 3

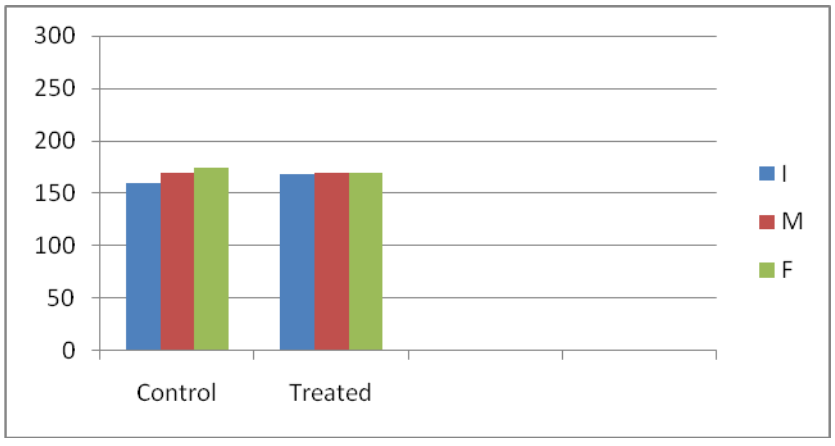


Figure No. 6: Change in body weight

6.1.2 Food and Water Observation

In general food and water particularly, feed intake was found to be increased during 14 days observational period but the changes were not remarkable as compared to control group.

Table no. 2: Effect of PM root Extract suspension 2000 mg/kg on feed and water intake

Group	Food (gm.)				Water (ml)			
	Initial	Middle	Final	Mean	Initial	Middle	Final	Mean
Control	19	17	13	16.3±0.28	14	11	15	13.33±0.05
Treated	17	15	13	15±0.70	13	15	17	15±0.69

Mean ± S.E.M. for n= 3

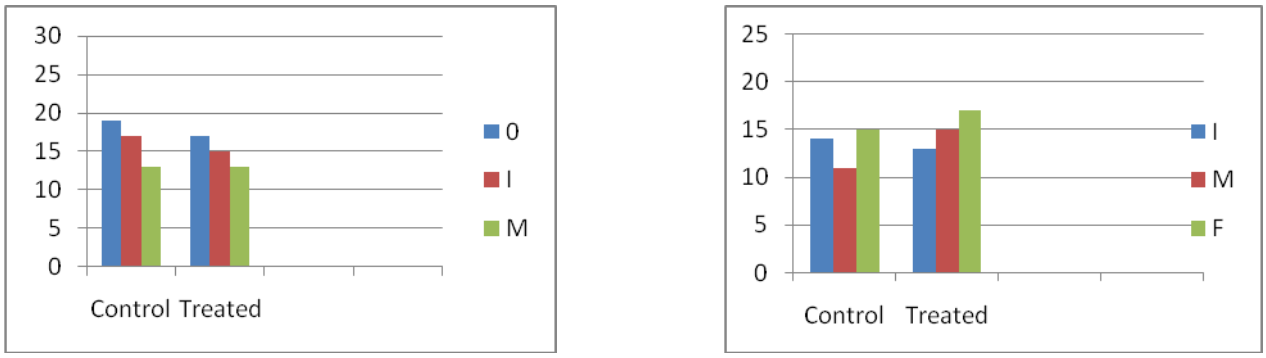


Figure No. 7: Change in Food and Water intake

6.1.3 Organ Weight Observation

Weight of vital organs of the animals was calculated. The effect of extract on principle organ weights relative to body water. The results revealed that, the essential organs such as kidneys, liver, heart, lung and spleen were not adversely affected throughout the treatment. Treated rat showed increased organ weight. Changes has been recorded in table.

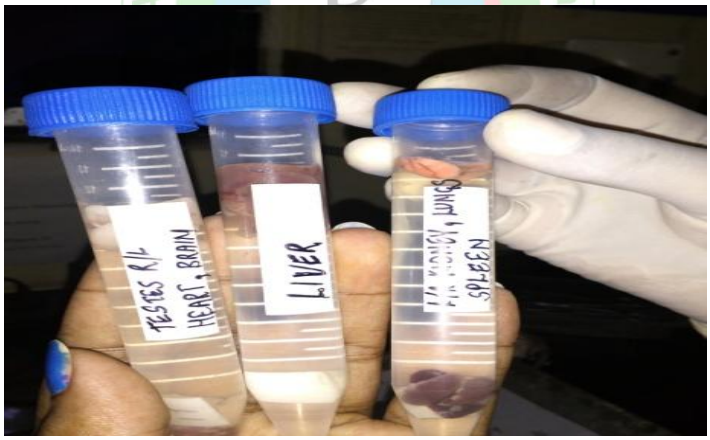


Figure no. 8: Weight of organs

Table No. 3: Effect of PM root extract suspension organ weight against control rats after treatment of 14 days.

S. N.	Organ	Control	Treated
1	Liver	02.82±0.05	2.91±0.08
2	Lung	0.94±0.18	0.87±0.12
3	Brain	1.01±0.21	1.10±0.13
4	Spleen	0.24±0.05	0.30±0.05
5	Heart	0.35±0.04	0.35±0.06
6	Kidney	0.35±0.08	0.33±0.08
7	Testis	0.37±0.06	0.14±0.06

Mean ±S.E.M. for n=3

6.1.4 Behavior Parameters

Table No. 4: Different behavior parameters of animal in toxicity study for 14 days

Days	30 Min			4Hrs			24 Hrs			48 Hrs			1week			2week		
Rat	**H**	**B**	**T**	**H**	**B**	**T**	**H**	**B**	**T**	**H**	**B**	**T**	**H**	**B**	**T**	**H**	**B**	**T**
Appearance	Normal			Unusual			Unusual			Normal			Unusual			Normal		
Activity	Unusual			Unusual			Unusual			Normal			Normal			Normal		
Fur coat	Unusual			Unusual			Normal			Normal			Normal			Normal		
Mucus membrane	Normal			Some Redness			Normal			Normal			Normal			Normal		
Body orifice	No			Discharge			No			No			No			No		
Eye lacrimation	Normal			Redness with Lacrimation			Redness			Normal			Normal			Normal		
Pupil Size	Normal			Pupils Dilated			Pupils Dilated			Normal			Normal			Normal		
Urination	Normal			Unusual			Unusual			Unusual			Unusual			Normal		
Respiration	Normal			Unusual			Unusual			Normal			Unusual			Normal		
Lethargy	Yes			Yes			Normal			Normal			Yes			Normal		
Convulsion	No			No			No			No			No			No		
Sedation	Normal			Yes			Normal			Normal			Normal			Normal		
Salivation	Normal			Yes			Normal			Normal			Normal			Normal		
Coma	No			No			No			No			No			No		
Mortality	No			No			No			No			No			No		

6.1.5 Hematological Analysis

Hematological values measured showed elevation of lymphocytes level, Hb and WBC level in treatment group. The value of MCV was significant increased as compared with the control group. Other hematology values, RBC_S, MCH, MCHC, Lymphocyte number and Platelet were not significantly different as compared to the control rats and they remained within normal limits (control values). Hematology data are presented in table form:

Table: 5 Effect of PM root Extract suspension 2000 mg/kg on Hematological Parameters

S.No.	Blood Parameters	Control Group	Treated Group
1	Hemoglobin	12.32±0.13	13.4±0.18
2	Total Leucocytes Count	11503±44.53	12545±17.23
3	**Differential Leukocytes Count**		
I	Granulocytes	15.64±1.32	23.30±2.32
II	Lymphocytes	82.30±1.57	62.63±2.41
III	Monocytes	0.02±0.70	1.66±0.15
IV	RBC Count	4.82±0.064	4.22±0.061
V	Platelet count	4.3±0.12	4.16±0.205
VI	MCV	67.11±1.65	81.91±0.36
VII	MCH	31.8±0.37	31.76±0.32
VII	MCHC	25.50±0.235	31±0.23
IX	PVC	39±0.36	43±0.43

Values represented Mean±SEM, N=3 (Student t-test)

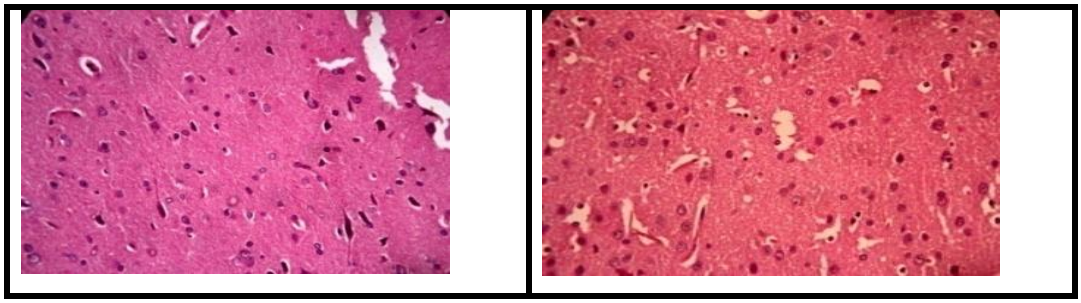
TLC: Total leukocyte count**, DLC:** Differential leukocyte count**, RBC:** Red blood cells, **MCV:** Mean corpuscular volume, **PCV:** Packed cell volume**, MCHC:** Mean corpuscular hemoglobin concentration**, PCV**: Packed cell volume.

6.1.6 Histopathology

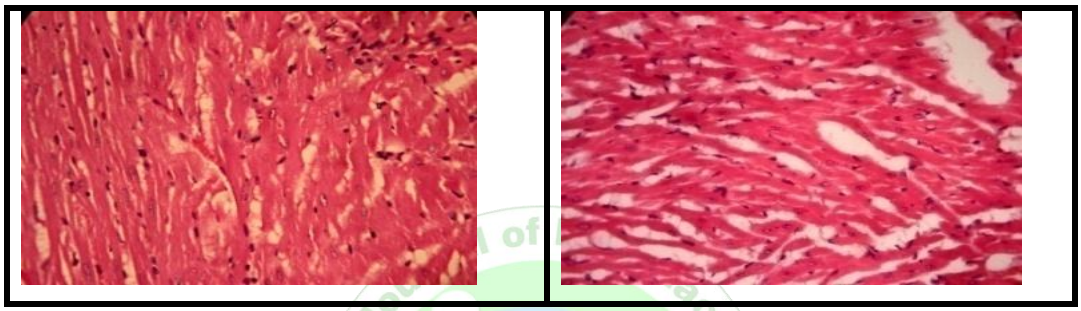
Macroscopic examination of the organs of the animals treated with PM root Extract suspension at a dose levels 2000mg/kg showed no changes in color compared to control. Autopsy at the end of the experimentation stage exposed no visible changes in the liver, kidney, lungs,

heart, brain and spleen from both control and treated rat in the histopathology analysis. The microscopic examination revealed that, all the organs from the treated rat did not show any alteration in cell in treated organs was more or less similar compared with the control organs.

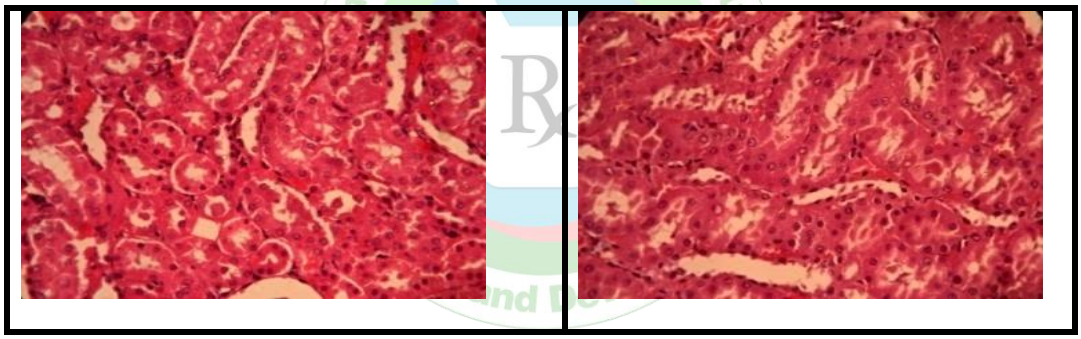
CONTROL **TREATED**



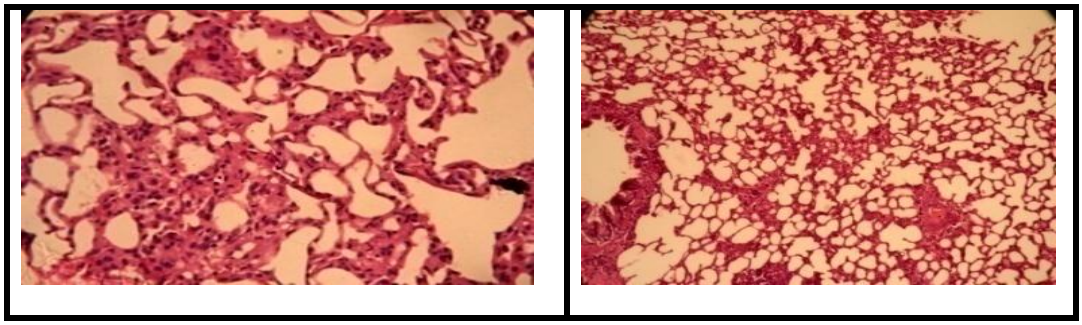
BRAIN



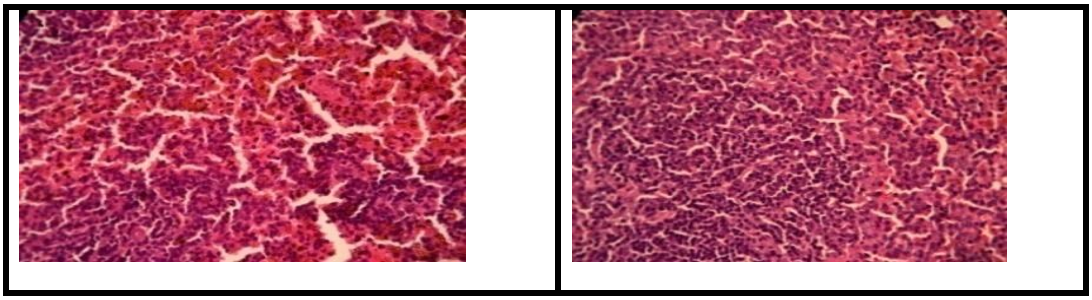
HEART



KIDNEY



LUNGS



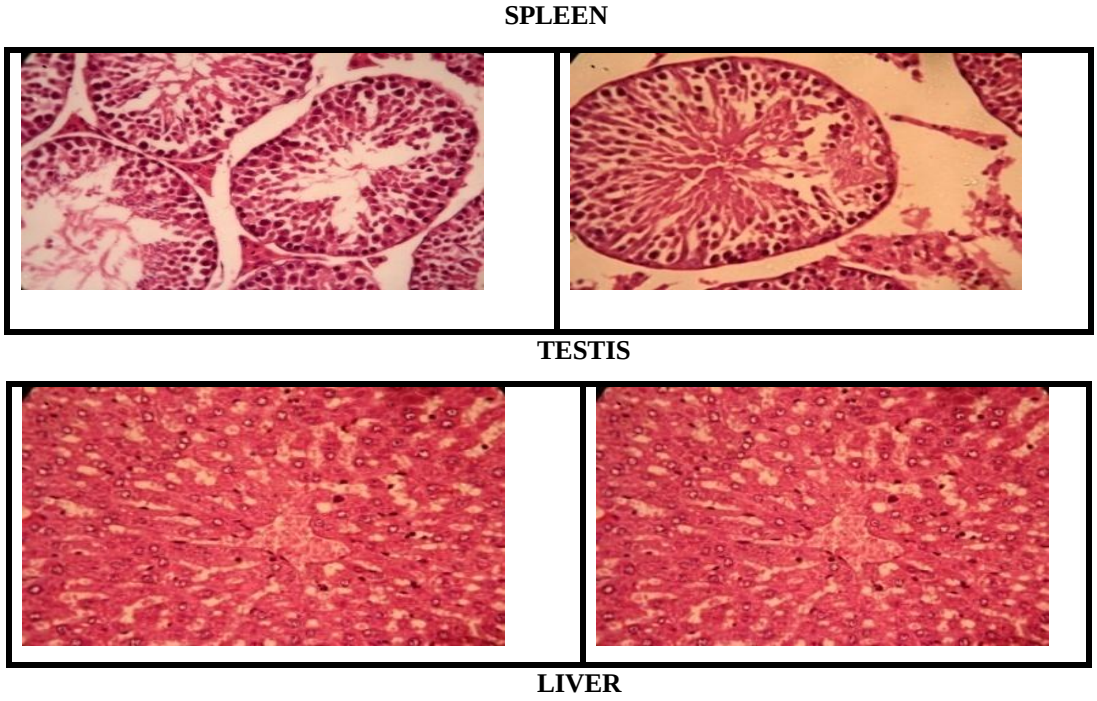


Figure No. 9: T.S of different organs of control and treated group at resolution of 40X

6.2 ANTISTRESS ACTIVITY

Following results were obtained in different model of screening Antistress drug.

6.2.1 Forced Swim Test

In this test animals treated with PM root Extract suspension(100 mg/kg, 200 mg/kg, 400 mg/kg) showed a decrease in their immobility times which was significant (84.83±2.17, 59.67±1.78 and 47.76±2.39 respectively; p<0.001) when compared with control (150.17±4.9). Similarly, animals treated with diazepam (1mg/kg), as expected, showed a significant decrease in the immobility time (38.5±2.89; p<0.001). (Fig no. 3.6)

Table No. 6: Effect of PM root Suspension on immobility time in FST

S.N.	Group	Drug Treatment	Duration of immobility(sec)
1	Control	Normal saline	149±4.9
2	Test gp 1st (low dose)	PM root Suspension (100mg/kg)	84.83±2.17***
3	Test gp 2nd (mid dose)	PM root Suspension (200mg/kg)	59.67±1.78***
4	Test gp 3rd (high dose)	PM root Suspension (400mg/kg)	47.76±2.39***
5	Standard	Diazepam (1mg/kg)	38.5±2.89***

6.2.2 Tail Suspension Test

In this test animals treated with PM root Suspension leaf(100 mg/kg, 200 mg/kg, 400 mg/kg) showed a decrease in their immobility times which was significant (135.33±3.19, 113.17±2.81 and 99.17±2.45 respectively; p<0.001) when compared with control (161.17±3.52). Similarly, animals treated with diazepam (1mg/kg), as expected, showed a significant decrease in the immobility time (73.33±2.11; p<0.001). (Fig no. 3.7)

Table 7: Effect of PM root Suspension on immobility time in TST

S.N.	Group	Drug Treatment	Duration of immobility(sec)
1	Control	Normal saline	161.17±3.52
2	Test gp 1st(low dose)	PM root Suspension (100mg/kg)	135.33±3.19***
3	Test gp 2nd(mid dose)	PM root Suspension (200mg/kg)	113.17±2.81***
4	Test gp 3rd(high dose)	PM root Suspension (400mg/kg)	99.17±2.45***
5	Standard	Diazepam (2mg/kg)	73.33±2.11***

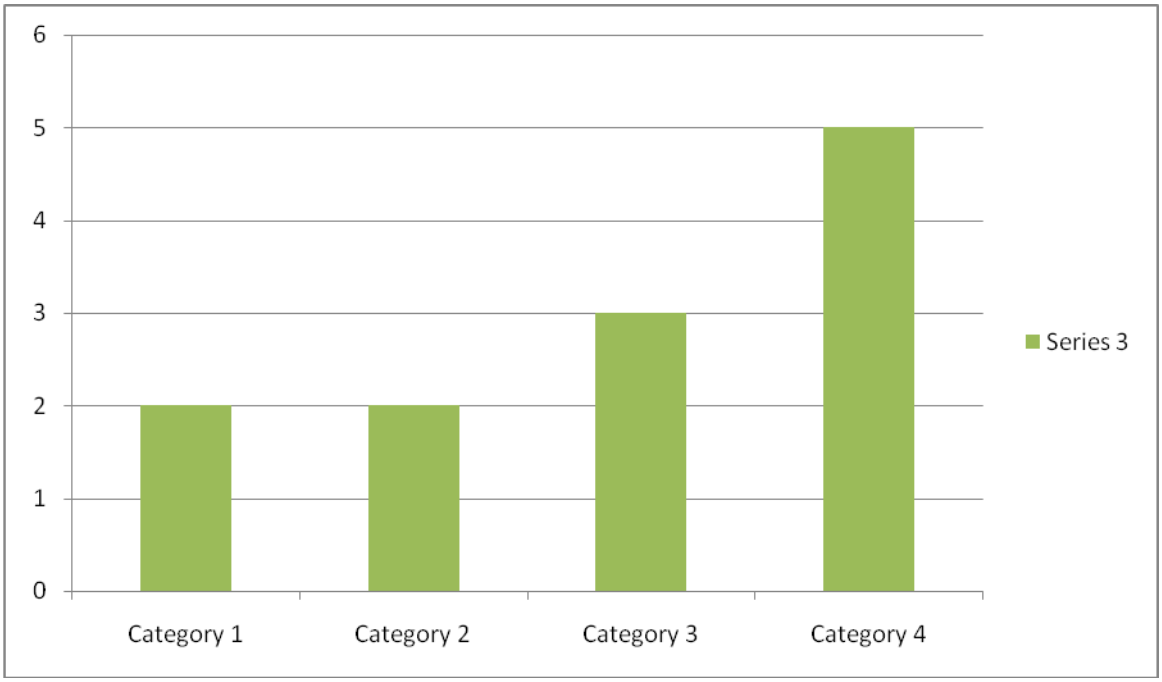


Figure No. 10: Effect of PM root Suspension and diazepam on duration of immobility time in FST. Results are expressed as mean±S.E.M (n=5). ***P<0.001 as compared to respective control group.

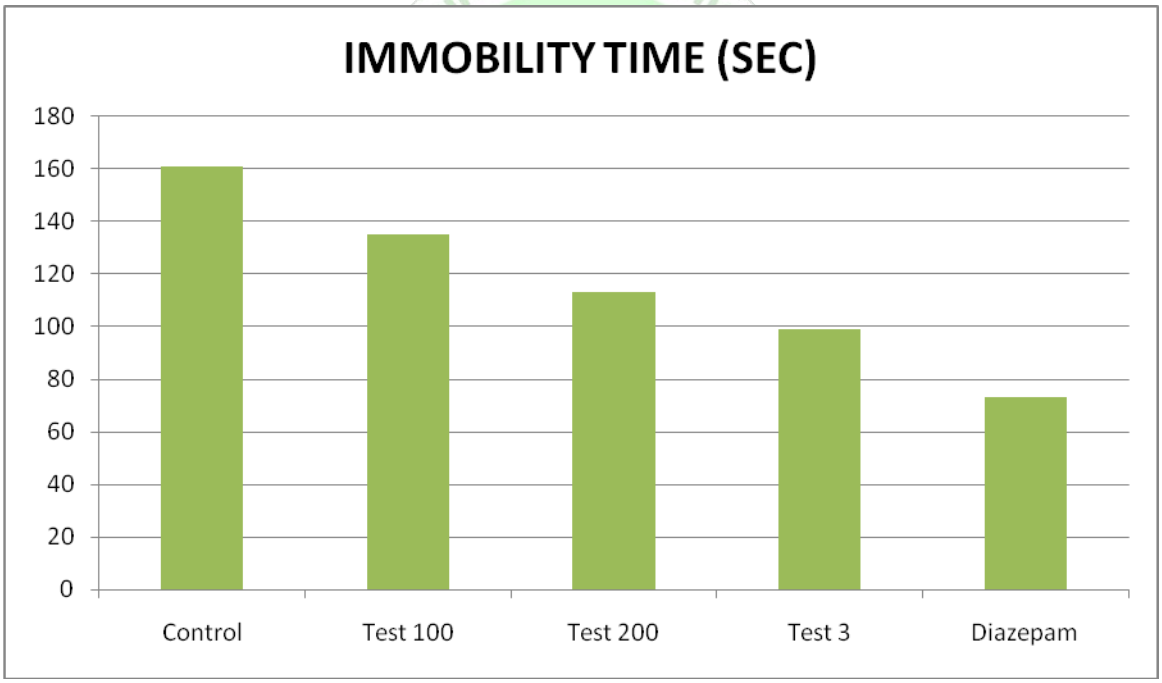


Figure no. 11: Effect of PM root Suspension and diazepam on duration of immobility time in TST. Results are expressed as mean±S.E.M (n=5). ***P<0.001 as compared to respective control group.

6.2.3 Anoxia Stress Tolerance Test

The results obtain from the anoxia stress tolerance test was articulated as Mean±SEM. Anoxia stress tolerance time was significantly (P<0.05) enhanced on 7^{th}, 14^{th}, 21^{st} in PM root Suspension (400 mg/kg) and Diazepam (1mg/kg) treated groups. There was increased anoxia tolerance time also seen after 2^{nd} & 3^{rd} week of PM root Suspension (200 mg/kg) treated group but not statistically significant result was obtained on 7^{th} day.

However, the effect of PM root Suspension (400 mg/kg) on anoxia stress tolerance time in mice was not statistically significant at the end of 1^{st}, 2^{nd}, 3^{rd} week of treatment.

Table No. 8: Effect of PM root Suspension on immobility time in Anoxia stress Tolerance test

Treatments groups	Duration of anoxia stress tolerance in minutes		
	First week	Second week	Third week
Control group (normal saline)	26±1.79	29.16±3.49	31.32±2.65
PM Susp. (100 mg/kg), p.o	38.45±4.62	43.33±3.42*	47±4.11*
PM Susp. (200 mg/kg), p.o	46.5±3.27**	46.33±4.34*	48.31±3.61**
PM Susp. (400 mg/kg), p.o	29.33±1.23	35.61±4.20	31.33±2.23***
Diazepam (1mg/kg), i.p	48.16±3.11***	48±3.43**	44.83±2.12**

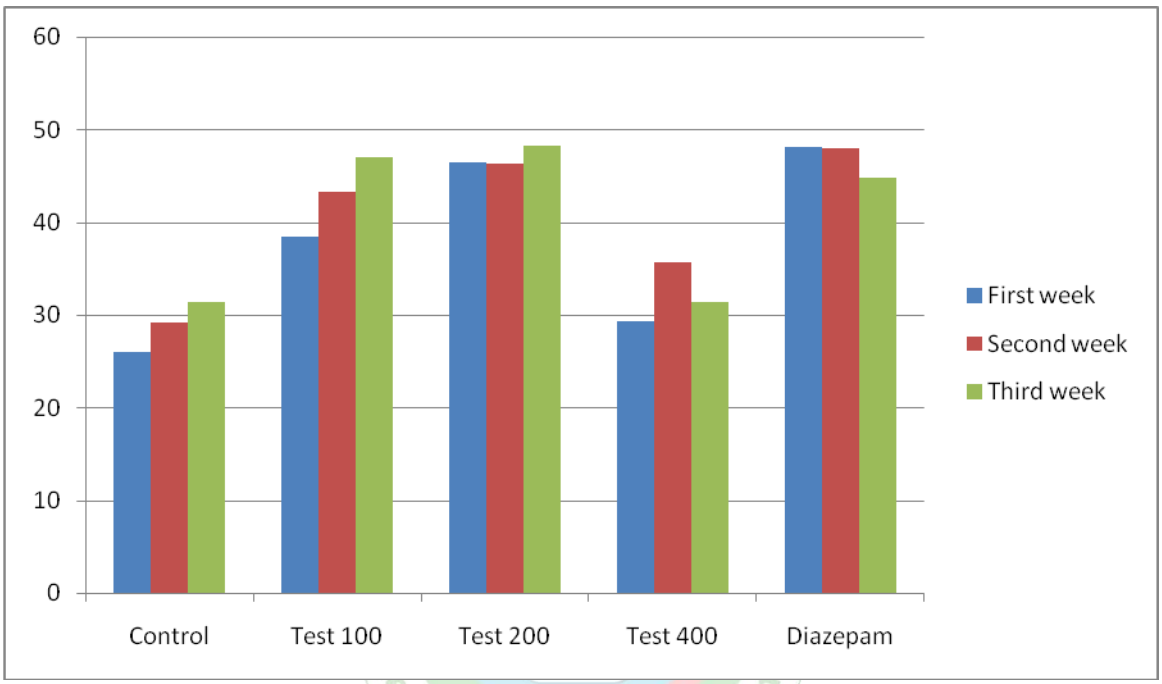


Figure no. 12: Effect of PM Susp.immobility time in Anoxia stress tolerance time.Values are expressed as Mean±SEM, n=5, analyzed by one way ANOVA followed by Dunnett s test, *Represent statistical significance vs. control ($p<0.05$)

DISCUSSION AND CONCLUSION

In modern era stress and stress related disorder are a significance cause of disease and contributing perhaps 75% of all illness. Stress defined as physical and psychological modification that disrupts the homeostasis and the balance of organism. Stress causes due to physical stressor like loud noise, big crowds and cluttered surroundings. Stress is a non-particular reaction of the body recognized to alter physiological homeostasis of the organism resulting in various neuronal, endocrine and primitive dysfunctions.

Piper methysticum root Extract shows the antistress activity. *Piper methysticum* root Extract used as the respiratory disorders like asthma, allergy. The mucolytic (reducing the viscosity of mucus) and bronchodilator (decreasing resistance in the breathing airways) properties of *Piper methysticum* root Extract are helpful in liquefying and relieving nasal and bronchial congestion. *Piper methysticum* root Extract proving to be used an antistress but there is no scientific data available.

Studies with Forced Swim Test induced stress-It has been reported that stress can be induced by Mice was individually forced to swim in open glass compartment (25cm x15 cm x 25) containing fresh water to a height of 15cm and maintained at 26 degree. At this height of water and animals were not able to support themselves by touching the bottom or the side walls of chamber with their hind paws or tail. Water in the chamber was changed after subjecting each animal to FST because used water has been to show the alter behavior. Each one animal shows energetic movement during first 2 min period of the test. The duration of immobility was manually record during the next 4 min of the total 6 min testing. In this test animals treated with *Piper methysticum* root Extract (100 mg/kg, 200 mg/kg, 400 mg/kg) showed a decrease in immobility time as compared to control. Standard drug diazepam also shown a decrease in immobility time.

Studies with Tail Suspension Test stress model-Tail suspension test commonly employed behavioral model for screening antistress like activity in rodents was first proposed by steru[17] The animal were moved from housing colony to laboratory in their own cages and allowed to adapt to laboratory conditions for 1-2 hr. Each animal were individually suspended to the edge of table, 50cm above the floor by adhesive tape placed approx 1cm from the tip of the tail. Each animal under test was both acoustically and visually isolated from other animals during the test. The total period of immobility was recorded manually for 6 min. Animal was considered to immobile when it did not

show any body movement, hung passively and completely motionless. The test was conducted in dim lighted room and each mouse was used only once in the test. The observer, recording the immobility of animals was blind to drug treatments given to the animals under study. A decrease in immobility time was observed in Tail Suspension Test in both treated groups. This increase was significant when compared with control group.

Studies with Anoxia Stress Tolerance Test

Swiss mice of either sex were divided into 5 groups, each gp contain 5 mice. Group 1st serve as normal saline, group 1st, 2nd, 3rdwas treated with *Piper methysticum* root extract at doses 100, 200, 400mg/kg, p.o and stress. Group 5th mice were treated with diazepam (1mg/kg) and stress. The drug treatment was carried out daily for a period of 21 days. At the end of this week i.e. 1st, 2nd, 3rd weeks of drug treatment, the animal was exposed to anoxia tolerance time was noted. Hermetic vessel of 1 liter air capacity was used to provoke to anoxia stress. Each one animal was kept in the hermetic vessel and the time to show the first sign of seizure was noted and were immediately removed from the vessel and resuscitated if needed. The results obtained from the anoxia stress tolerance test was expressed as Mean+-SEM. Anoxia stress tolerance time was significantly ($P<0.05$) enhanced on 7th , 14th , 21st in *Piper methysticum* root Extract (400 mg/kg) and Diazepam (1mg/kg) treated groups. There was increased anoxia tolerance time also seen after 2nd & 3rd week of *Piper methysticum* root Extract (200 mg/kg) treated group but not statistically significant result was obtained on 7th day. However, the effect of Piper methysticum root Extract (400 mg/kg) on anoxia stress tolerance time in mice was not statistically significant at the end of 1st, 2nd, 3rd week of treatment.

CONCLUSION

It can be accomplished from the current study that given stress by FST, TST & Anoxia stress tolerance test model, animal was treat with three doses of *Piper methysticum* root Extract (100, 200, 400 mg/kg, p.o) showed decreases in their immobility times when compared with control. In the same way, animals treat with diazepam (1mg/kg) as predictable, show a major decrease in the immobility time. The current study investigation discovered that Piper methysticum root Extract shows antistress activity by prevent stress induce by FST, TST& Anoxia stress tolerance test model.

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